

First-passage problems in DNA replication: effects of template tension on stepping and exonuclease activities of a DNA polymerase motor

Ajeet K. Sharma and Debashish Chowdhury
Department of Physics, Indian Institute of Technology,
Kanpur, 208016

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Abstract

A DNA polymerase (DNAP) replicates a template DNA strand. It also exploits the template as the track for its own motor-like mechanical movement. In the polymerase mode it elongates the nascent DNA by one nucleotide in each step. But, whenever it commits an error by misincorporating an incorrect nucleotide, it can switch to an exonuclease mode. In the latter mode it excises the wrong nucleotide before switching back to its polymerase mode. We develop a stochastic kinetic model of DNA replication that mimics an *in-vitro* experiment where a single-stranded DNA, subjected to a mechanical tension F , is converted to a double-stranded DNA by a single DNAP. The F -dependence of the average rate of replication, which depends on the rates of both polymerase and exonuclease activities of the DNAP, is in good qualitative agreement with the corresponding experimental results. We introduce 9 novel distinct *conditional dwell times* of a DNAP. Using the methods of first-passage times, we also derive the exact analytical expressions for the probability distributions of these conditional dwell times. The predicted F -dependence of these distributions are, in principle, accessible to single-molecule experiments.

1 Introduction

A linear molecular motor is either a macromolecule or macromolecular complex that moves along a filamentous track [1, 2, 3, 4]. In spite of its noisy stepping kinetics, on the average, it moves in a directed manner. Its mechanical work is fuelled by the input energy which, for many motors, is chemical energy. The distributions of the dwell times of a motor at discrete positions on its track as well as the duration of many complex motor-driven intracellular processes have been calculated [5, 6, 7, 8, 9, 10, 12] using the methods of first-passage times [13], a well-known formalism in non-equilibrium statistical mechanics. Experimentally measured distributions of dwell times of a motor can be utilized to extract useful information on its kinetic scheme [10, 14].

For motors which can step both forward and backward on a linear track, four distinct conditional dwell times can be defined; distributions of these four

conditional dwell times have been calculated for some motors [7, 8, 11, 12]. In this paper we consider a specific molecular motor called DNA polymerase (DNAP) and argue that its movements on the track is characterized by *nine* distinct conditional dwell times because of the coupling of its dual roles during its key biological function. We define these nine conditional dwell times and calculate their distributions analytically treating each of these as an appropriate first-passage time. As a byproduct of this exercise, we obtain an important result that characterizes one of its average properties; the predicted behaviour is consistent with the corresponding experimental observations reported earlier in the literature. The distributions of the nine conditional dwell times are new predictions which, we believe, can be tested by single-molecule experiments.

Deoxyribonucleic acid (DNA) is a polynucleotide, i.e., a linear heteropolymer whose monomeric subunits are drawn from a pool of four different species of nucleotides, namely, A (Adenine), T (Thymine), C (Cytosine) and G (Guanine). In this heteropolymer the nucleotides are linked by phosphodiester bonds. The genetic message is chemically encoded in the sequence of the nucleotide species. DNA polymerase (DNAP) [15], the enzyme that replicates DNA, carries out a template-directed polymerization [16]. During this processes, repetitive cycles of nucleotide selection and phosphodiester bond formation is performed to polymerize a DNA strand. In every elongation cycle, hydrolysis of the substrate molecule supplies sufficient amount of energy to the DNAP for performing its function. Therefore, DNAPs are also regarded as molecular motor [1, 2, 3, 4] that transduce chemical energy into mechanical work while translocating step-by-step on the template DNA strand that serves as a track for these motors.

In an *in-vitro* experiment, Wuite et al. [17] applied a tension on a ssDNA. The two ends of this DNA fragment were connected to two dielectric beads; one end was held by micro-pipette, while the other end, trapped optically by a laser beam, was pulled. This DNA fragment also served as a template for the replication process carried out by a DNAP. Replication converted the ssDNA into a dsDNA. The average rate of replication was found to vary *nonmonotonically* with the tension applied on the template strand [17]. Similar results were obtained also in the experiments carried out by Maier et al. [18], where magnetic tweezers were used to apply the tension on template DNA. The observed nonmonotonic variation of the average rate of replication was explained [17, 18, 19, 20, 21] as a consequence of the difference in the force-extension curves of ssDNA and dsDNA [22].

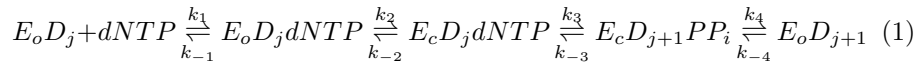
Operation of a DNAP is error-prone. But, unless corrected, replication error would result in a defective genome for the daughter cells [?]. Therefore, DNAP is capable of correcting most of its own error during the ongoing replication process itself. A DNAP performs its normal function as a polymerase by catalyzing the elongation of a new ssDNA molecule using another ssDNA as a template. However, upon committing a misincorporation of a nucleotide in the elongating DNA, the DNAP can detect its own error and transfer the nascent DNA to another site where it catalyses excision of the wrongly incorporated nucleotide. The distinct sites, where the polymerisation (pol) and exonuclease (exo) reactions are catalyzed, are separated by 3-4 nm on the same DNAP [24]. The nascent DNA is transferred to the pol site from the exo site after the wrong nucleotide is cleaved from its tip by the DNAP. Thus, the transfer of the DNA between the pol and exo sites couples the polymerase and exonuclease activities of the DNAP.

In the next section we develop a microscopic model for the replication of a ssDNA template that is subjected to externally applied tension F , a situation that is very similar to the *in-vitro* experiment reported in refs. [17, 18]. The rates of both pol and exo activities of the DNAP enter into the expression that we derive for the average rate of elongation of the DNA. The F -dependence of this rate is consistent with the experimental observations reported in [17, 18]. We then define 9 distinct conditional dwell times of the DNAP and identifying each of these with an appropriate first-passage time [13], we calculate their distributions analytically. We believe that experimental measurements of these distributions are likely to elucidate the nature of the interplay of the pol and exo activities of DNAP.

2 Model

The nucleotides on the template DNA are labelled sequentially by the integer index j ($j = 1, 2, \dots, L$) which also serves to indicate the position of the DNAP on its track. The chemical (or conformational) state of the DNAP is denoted by a discrete variable μ ($\mu = 1, 2, \dots, 5$). The state of the DNAP during replication is described by the pair j, μ . The kinetic scheme used for our model is adapted from that proposed originally by Patel et al. [25] and subsequently utilized by various other groups [26, 20]. The kinetic scheme of our model is shown in figure (1), where the four different values 1, 2, 3 and 4 of μ are the allowed chemical states in the polymerase-active mode of the enzyme, while in chemical state 5 the exonuclease catalytic site is activated.

The structure of DNA polymerase resembles a “cupped right hand” of a human, where its sub domains are recognized as palm, thumb and finger sub domains [27]. Template DNA enters from the finger sub-domain and takes exit from thumb sub-domain. The catalytic site where the binding occurs is located between finger and palm domain. Transitions between polymerase activated kinetic states of the enzyme (i.e., chemical states 1, 2, 3 and 4) can be summarized as [28, 29]



where E_c and E_o represent the closed and open finger configuration DNAP, respectively, while D_j denotes the length of the nascent DNA strand.

Let us start with the state $E_o D_j$, labelled by $\mu = 1$, in which the finger domain of DNAP is open and the DNAP is located at the site j on its template. Now a substrate molecule (dNTP) binds with the DNAP and resulting state $E_o D_j dNTP$ is labeled by “2”. The transition $1 \rightarrow 2$ take place with rate k_1 , while corresponding reverse transition $2 \rightarrow 1$ occurs with rate k_{-1} . Binding energy of dNTP switches the open finger configuration of DNAP into closed finger configuration and the corresponding transition 2 ($E_o D_j dNTP$) \rightarrow 3 ($E_c D_j dNTP$) take place at the rate k_2 . The reverse transition 3 \rightarrow 2 occurs at the rate k_{-2} . This new closed finger configuration of DNAP catalyzes the formation of phosphodiester bond between dNTP and nascent DNA strand thereby elongating the nascent DNA from length j to $j + 1$; this process is represented by the transition 3 ($E_c D_j dNTP$) \rightarrow 4 ($E_c D_{j+1} PP_i$) that occurs at the rate k_3 (k_{-3} being the rate of the reverse transition). Finally, the transition

$4(j) \rightarrow 1(j+1)$ completes one elongation cycle; the corresponding rates of the forward and reverse transitions are k_4 and k_{-4} , respectively. The transition $4(j) \rightarrow 1(j+1)$ captures more than one sub-step which includes opening of the finger domain, release of PP_i and the forward movement of the DNAP to the next site on the template.

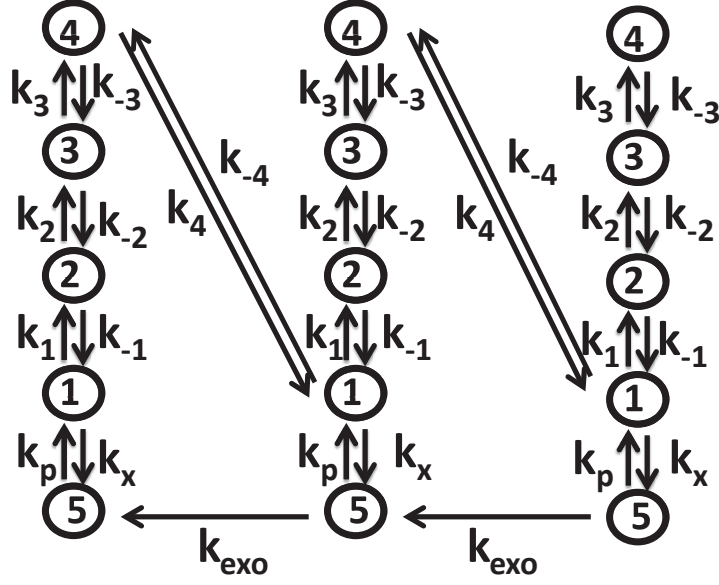


Figure 1: A pictorial depiction of 5 state kinetic model for DNA polymerase (see the text for a detailed explanation).

Immediately after completing one elongation cycle, the DNAP is normally ready to bind with a new substrate molecule and initiate the next elongation cycle. However, if a wrong nucleotide is incorporated in an elongation cycle, the DNAP is likely to transfer the nascent DNA from the pol site to the exo site. This switching from pol to exo activity is represented by the transition $1 \rightarrow 5$ which occurs at the rate k_x ; the reverse transition, without cleavage, takes place at the rate k_p . In the exo mode the cleavage of the last incorporated nucleotide, at the rate k_{exo} , effectively alters the position of the DNAP from $j+1$ to j .

2.1 Force dependent chemical steps

Not all the rate constants change with the tension F applied on the template. Tension-dependence of those rate constants ω which are influenced by the tension F are assumed to have the form [19]

$$\omega(F) = \omega(0)e^{-\theta\Delta\Phi(F)/k_B T} \quad (2)$$

where θ is a phenomenological parameter, k_B is the Boltzmann constant, T is the absolute temperature and $\Delta\Phi(F)$ is the free energy difference between the corresponding initial and final states. In our model $\Delta\Phi(F)$ is the difference

between the free energies of a single base pair in a dsDNA and that in a ssDNA. The details of this calculation are given in Appendix A.

Now we hypothesize that only the following transitions are affected by the tension F : (I) $3 \rightarrow 4$, i.e., the polymerization step, where new dNTP subunit is incorporated into nascent DNA chain and a single stranded nucleotide is converted into a double stranded DNA. Negative value of $\Delta\Phi(F)$ favours the incorporation of substrate molecule whereas positive value of $\Delta\Phi(F)$ disfavours the transition.

(II) $1 \rightarrow 5$ i.e., the transfer of the nascent DNA from the pol site to the exo site of the DNAP. These two catalytic sites are separated by $3.5A^0$ and a transfer of the nascent DNA between them includes major change in the DNAP conformation that involves a β hairpin [30, 31, 32]. Moreover, polymerase to *exonuclease* switching causes local melting of the dsDNA. Depending upon the sign of $\Delta\Phi(F)$, tension applied on the template strand may favour or disfavour the $1 \rightarrow 5$ transition.

As we show in the next section, following force dependence of $k_3(F)$ and $k_x(F)$ shows a good qualitative agreement with the experimental data.

$$k_3(F) = k_3(0)\exp(-3\Delta\Phi(F)/K_BT) \text{ and } k_x(F) = k_x(0)\exp(3\Delta\Phi(F)/K_BT) \quad (3)$$

3 Results

3.1 Force velocity curve

In this subsection we derive the force-velocity curve for our model DNAP motor and compare it with those reported earlier in the literature. Let $P_\mu(j, t)$ be the probability of finding DNAP in chemical state μ , at the position j on its track, at time t . The probability to finding the DNA polymerase in chemical state μ , irrespective of its position, is

$$P_\mu(t) = \sum_{j=1}^L P_\mu(j, t) \quad (4)$$

where L is the total number of nucleotides in template DNA strand. Normalisation of the probability imposes the condition

$$\sum_{\mu=1}^5 P_\mu(t) = 1 \quad (5)$$

at all times. The time evolution of the probability $P_\mu(t)$ is governed by following equations

$$\frac{dP_1(t)}{dt} = -(k_x + k_1 + k_{-4})P_1(t) + k_{-1}P_2(t) + k_4P_4(t) + k_pP_5(t) \quad (6)$$

$$\frac{dP_2(t)}{dt} = k_1P_1(t) - (k_{-1} + k_2)P_2(t) + k_{-2}P_3(t) \quad (7)$$

$$\frac{dP_3(t)}{dt} = k_2P_2(t) - (k_{-2} + k_3)P_3(t) + k_{-3}P_4(t) \quad (8)$$

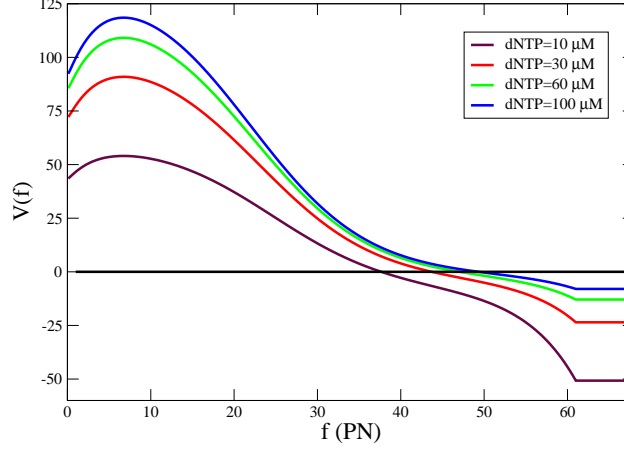


Figure 2: Velocity of DNA polymerase is plotted against the force applied on template strand for a few different values of dNTP concentration. The numerical values of the parameters used for this plot are listed in table 1.

$$\frac{dP_4(t)}{dt} = k_{-4}P_1(t) + k_3P_3(t) - (k_{-3} + k_4)P_4(t) \quad (9)$$

$$\frac{dP_5(t)}{dt} = k_xP_4(t) - k_pP_5(t) \quad (10)$$

Now we solve these equations in steady state and calculate the probability of finding the DNA polymerase in μ th chemical state (P_μ^{st}).

$$P_\mu^{st} = \frac{x_\mu}{x_1 + x_2 + x_3 + x_4 + x_5} \quad (11)$$

Expressions for x_μ 's are given in Appendix B.

Now we define the average rate of polymerization V_p and the average rate of excision V_e as

$$V_p = P_1^{st}k_1 - P_2^{st}k_{-1} \text{ and } V_e = k_{exo}P_5^{st} \quad (12)$$

Therefore, the average velocity of the DNAP on its track is

$$V = V_p - V_e \quad (13)$$

In figure (2) the average velocity of the DNAP is plotted against the tension applied on DNA track. Rate constants used for this plot are collected from the literature [25] and listed in table 1.

Because of the F -dependence of the form assumed in (3), at lower tension transition $2 \rightarrow 3$ is rate limiting while at higher values of tension $3 \rightarrow 4$ becomes the rate limiting step. Frequent *poly* \rightarrow *exo* switching cause the significant increase in the exonuclease cleaving at higher forces. Observed trend of variation of the average velocity is the direct consequence of the nonmonotonic behavior of the $\Delta\Phi(F)$, shown in figure (5).

Rate constant	Numerical value
k_1	$50 \mu M^{-1} s^{-1}$
k_{-1}	$1000 s^{-1}$
k_2	$300 s^{-1}$
k_{-2}	$100 s^{-1}$
$k_3(0)$	$9000 s^{-1}$
k_{-3}	$18000 s^{-1}$
k_4	$600 s^{-1}$
k_{-4}	$25 s^{-1}$
$k_x(0)$	$.2 s^{-1}$
k_p	$700 s^{-1}$
k_{exo}	$900 s^{-1}$

Table 1: Numerical values of the rate constants used for graphical plotting of some typical curves obtained from the analytical expressions derived in this paper.

3.2 Distributions of dwell times and exonuclease turnover times

The average velocity of a DNAP and its dependence on the tension applied on the corresponding template does not provide any information on the intrinsic fluctuations in both the pol and exo activities of these machines. Probing fluctuations in the kinetics of molecular machines have become possible because of the recent advances in single molecule imaging, manipulation and enzymology. In this section we investigate theoretically how the fluctuations in the pol and exo activities of a DNAP would vary with the tension applied on the template DNA. For this purpose we use the same kinetic model introduced in section 1, that we have used in subsection 2.1 for calculating the average properties of DNAP.

The variable chosen to characterize the fluctuations in replication process is the time of dwell of DNAP at a single nucleotide on the template, which is nothing but the effective duration of its stay in that location. While moving on the one dimensional template strand three different mechanical steps are taken by DNAP, which are

- (1) Forward step in the pol mode: $4(j) \rightarrow 1(j+1)$.
- (2) Backward step in the pol mode: $1(j+1) \rightarrow 4(j)$.
- (3) Backward step (caused by cleavage) in the exo mode: $5(j+1) \rightarrow 5(j)$.

If a molecular motor takes more than one type of mechanical step then the fluctuations in the durations of its dwell at different locations cannot be characterized by a single distribution; instead, distributions of more than one type of conditional dwell times can be defined [9]. So, in the context of our model of DNAP, three different types of mechanical step would generate nine different distribution of conditional dwell times. We denote the forward, backward and cleavage steps are by the symbols $+$, $-$ and x , respectively. $\Psi_{mn}(t)$ is the conditional dwell time of the DNA polymerase when step m is followed by n , where the three allowed values of each of the subscripts m and n are $+$, $-$, x . For the convenience of calculation of the distributions $\Psi_{mn}(t)$, first we assume that the DNAP is already at the j_{th} site on the template strand and that the

rate constants for all the transitions leading to this special site j are equated to zero. In other words,

- (1) $k_4 = 0$ only for the transition $4(j-1) \rightarrow 1(j)$ (and not for any $i \neq j$),
- (2) $k_{-4} = 0$ only for $1(j+1) \rightarrow 4(j)$ (and not for any $i \neq j$),
- (3) $k_{exo} = 0$ only for $5(j+1) \rightarrow 5(j)$ (and not for any $i \neq j$).

Now appropriate initial conditions will ensure the type of previous step taken by DNAP.

If $P_\mu(j, t)$ is the probability of finding the DNA polymerase in μ_{th} chemical state at site j at time t , then time evolution of these probabilities are governed by following master equation.

$$\frac{dP_1(j, t)}{dt} = -(k_{-4} + k_1 + k_x)P_1(j, t) + k_{-1}P_2(j, t) + k_pP_5(j, t) \quad (14)$$

$$\frac{dP_2(j, t)}{dt} = k_1P_1(j, t) - (k_{-1} + k_2)P_2(j, t) + k_{-2}P_3(j, t) \quad (15)$$

$$\frac{dP_3(j, t)}{dt} = k_2P_2(j, t) - (k_{-2} + k_3)P_3(j, t) + k_{-3}P_4(j, t) \quad (16)$$

$$\frac{dP_4(j, t)}{dt} = k_3P_3(j, t) - (k_4 + k_{-3})P_4(j, t) \quad (17)$$

$$\frac{dP_5(j, t)}{dt} = k_xP_1(j, t) - (k_p + k_{exo})P_5(j, t) \quad (18)$$

These equation can be re-expressed in the following matrix form.

$$\frac{d}{dt}\mathbf{P}(\mathbf{t}) = \mathbf{M}\mathbf{P}(\mathbf{t}) \quad (19)$$

Here $\mathbf{P}(\mathbf{t})$ is a column matrix, whose elements are $P_1(j, t)$, $P_2(j, t)$, $P_3(j, t)$, $P_4(j, t)$ and $P_5(j, t)$. And

$$\mathbf{M} = \begin{bmatrix} -(k_{-4} + k_1 + k_x) & k_{-1} & 0 & 0 & k_p \\ k_1 & -(k_{-1} + k_2) & k_{-2} & 0 & 0 \\ 0 & k_2 & -(k_{-2} + k_3) & k_{-3} & 0 \\ 0 & 0 & k_3 & -(k_4 + k_{-3}) & 0 \\ k_x & 0 & 0 & 0 & -(k_p + k_{exo}) \end{bmatrix} \quad (20)$$

Now introducing the Laplace transform of the probability of kinetic states by,

$$\tilde{P}_\mu(j, s) = \int_0^\infty P_\mu(j, t)e^{-st}dt \quad (21)$$

Solution of equation (19) in Laplace space is,

$$\tilde{\mathbf{P}}(j, s) = (s\mathbf{I} - \mathbf{M})^{-1}\tilde{\mathbf{P}}(j, 0) \quad (22)$$

Here $\tilde{\mathbf{P}}(j, s)$ is the vector of the probability of individual chemical state in Laplace space and $\tilde{\mathbf{P}}(j, 0)$ is the column vector of initial probabilities.

Determinant of matrix $s\mathbf{I} - \mathbf{M}$ is a fifth order polynomial

$$\det(s\mathbf{I} - \mathbf{M}) = \alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta; \quad (23)$$

full expressions for α , β , γ , δ , ϵ and ζ in terms of the primary rate constants are given in Appendix C.

3.2.1 Calculation of $\Psi_{++}, \Psi_{+-}, \Psi_{+x}$

Following set of initial conditions guarantees that previous step taken by DNA polymerase is a forward step.

$$P_1(j, 0) = 1 \text{ and } P_2(j, 0) = P_3(j, 0) = P_4(j, 0) = P_5(j, 0) = 0 \quad (24)$$

So three different distribution of dwell time, where first step is forward, are defined as follows:

$$\Psi_{++}(t) = P_4(j, t)k_4|_{[P_1(j,0)=1, P_2(j,0)=P_3(j,0)=P_4(j,0)=P_5(j,0)=0]} \quad (25)$$

$$\Psi_{+-}(t) = P_1(j, t)k_{-4}|_{[P_1(j,0)=1, P_2(j,0)=P_3(j,0)=P_4(j,0)=P_5(j,0)=0]} \quad (26)$$

$$\Psi_{+x}(t) = P_5(j, t)k_{exo}|_{[P_1(j,0)=1, P_2(j,0)=P_3(j,0)=P_4(j,0)=P_5(j,0)=0]} \quad (27)$$

By applying the initial condition (24) in equation (22), we get

$$\tilde{P}_4(j, s) = \frac{a_0 + a_1 s}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (28)$$

$$\tilde{P}_1(j, s) = \frac{b_4 s^4 + b_3 s^3 + b_2 s^2 + b_1 s + b_0}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (29)$$

$$\tilde{P}_5(j, s) = \frac{c_3 s^3 + c_2 s^2 + c_1 s + c_0}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (30)$$

Mathematical expressions for $a_0, a_1, b_0, b_1, b_2, b_3, b_4, c_0, c_1, c_2$ and c_3 are given in Appendix D.

By inserting the inverse Laplace transforms of the expressions (28), (29) and (30) into the equations (25), (26) and (27), respectively, we get

$$\begin{aligned} \Psi_{++}(t) &= \left[\frac{(a_0 - a_1 \omega_1)k_4}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\ &+ \left[\frac{(a_0 - a_1 \omega_2)k_4}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\ &+ \left[\frac{(a_0 - a_1 \omega_3)k_4}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\ &+ \left[\frac{(a_0 - a_1 \omega_4)k_4}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\ &+ \left[\frac{(a_0 - a_1 \omega_5)k_4}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \end{aligned} \quad (31)$$

$$\begin{aligned} \Psi_{+-}(t) &= \left[\frac{(b_0 - b_1 \omega_1 + b_2 \omega_1^2 - b_3 \omega_1^3 + b_4 \omega_1^4)k_{-4}}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\ &+ \left[\frac{(b_0 - b_1 \omega_2 + b_2 \omega_2^2 - b_3 \omega_2^3 + b_4 \omega_2^4)k_{-4}}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\ &+ \left[\frac{(b_0 - b_1 \omega_3 + b_2 \omega_3^2 - b_3 \omega_3^3 + b_4 \omega_3^4)k_{-4}}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\ &+ \left[\frac{(b_0 - b_1 \omega_4 + b_2 \omega_4^2 - b_3 \omega_4^3 + b_4 \omega_4^4)k_{-4}}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\ &+ \left[\frac{(b_0 - b_1 \omega_5 + b_2 \omega_5^2 - b_3 \omega_5^3 + b_4 \omega_5^4)k_{-4}}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \end{aligned} \quad (32)$$

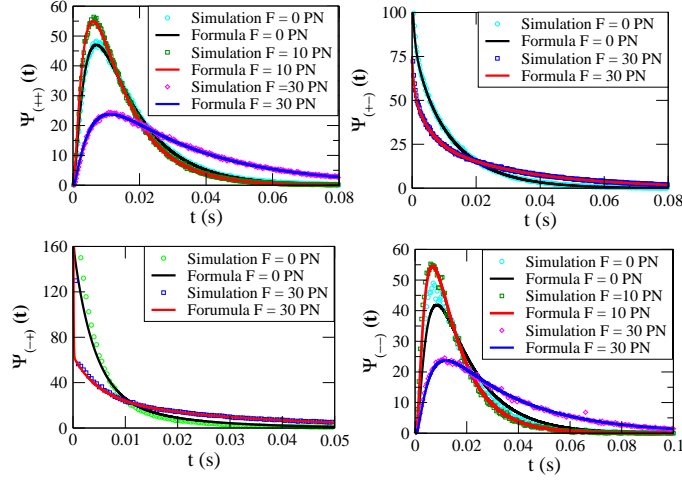


Figure 3: $\Psi_{++}(t)$, $\Psi_{+-}(t)$, $\Psi_{-+}(t)$, and $\Psi_{--}(t)$ are plotted against t for a few different values of F

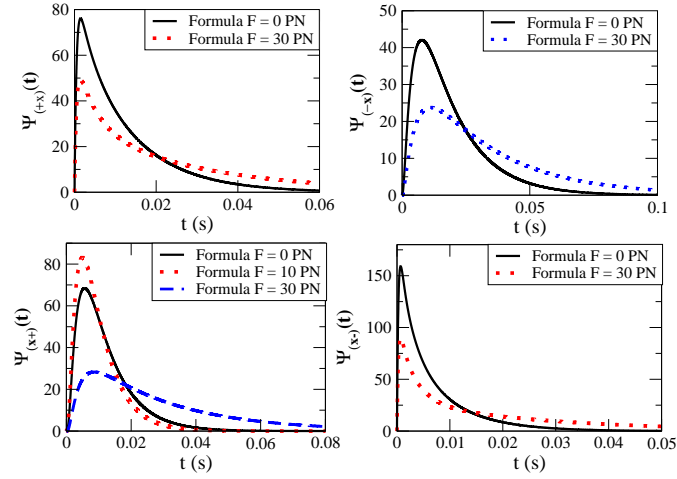


Figure 4: $\Psi_{+x}(t)$, $\Psi_{-x}(t)$, $\Psi_{x+}(t)$, and $\Psi_{x-}(t)$ are plotted against t for a few different values of F

$$\begin{aligned}
\Psi_{+x}(t) = & \left[\frac{(c_0 - c_1\omega_1 + c_2\omega_1^2 - c_3\omega_1^3)k_{exo}}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\
& + \left[\frac{(c_0 - c_1\omega_2 + c_2\omega_2^2 - c_3\omega_2^3)k_{exo}}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\
& + \left[\frac{(c_0 - c_1\omega_3 + c_2\omega_3^2 - c_3\omega_3^3)k_{exo}}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\
& + \left[\frac{(c_0 - c_1\omega_4 + c_2\omega_4^2 - c_3\omega_4^3)k_{exo}}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\
& + \left[\frac{(c_0 - c_1\omega_5 + c_2\omega_5^2 - c_3\omega_5^3)k_{exo}}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \quad (33)
\end{aligned}$$

where $\omega_1, \omega_2, \omega_3, \omega_4$ and ω_5 are the roots of following equation

$$\alpha\omega^5 - \beta\omega^4 + \gamma\omega^3 - \delta\omega^2 + \epsilon\omega - \zeta = 0; \quad (34)$$

the explicit expressions of $\alpha, \beta, \gamma, \delta, \epsilon$ and ζ in terms of the primary rate constants of the kinetic model are given in appendix C. The coupled nature of the pol and exo activities is revealed by the mixing of the corresponding rate constants in the expressions of $\Psi_{+, \pm}$ and Ψ_{+x} .

3.2.2 Calculation of $\Psi_{-+}, \Psi_{--}, \Psi_{-x}$

Following initial conditions ensures that DNA polymerase has reached to site j by making a backward step:

$$P_4(j, 0) = 1 \text{ and } P_1(j, 0) = P_2(j, 0) = P_3(j, 0) = P_5(j, 0) = 0 \quad (35)$$

So three different distributions of dwell time, where first step is backward, are defined as follows:

$$\Psi_{-+}(t) = P_4(j, t)k_4 |_{[P_4(j,0)=1, P_1(j,0)=P_2(j,0)=P_3(j,0)=P_5(j,0)=0]} \quad (36)$$

$$\Psi_{--}(t) = P_1(j, t)k_{-4} |_{[P_4(j,0)=1, P_1(j,0)=P_2(j,0)=P_3(j,0)=P_5(j,0)=0]} \quad (37)$$

$$\Psi_{-x}(t) = P_5(j, t)k_{exo} |_{[P_4(j,0)=1, P_1(j,0)=P_2(j,0)=P_3(j,0)=P_5(j,0)=0]} \quad (38)$$

After applying the above initial condition in equation (22), we get

$$\tilde{P}_4(j, s) = \frac{d_4 s^4 + d_3 s^3 + d_2 s^2 + d_1 s + d_0}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (39)$$

$$\tilde{P}_1(j, s) = \frac{k_x k_{-1} k_{-2} k_{-3}}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (40)$$

$$\tilde{P}_5(j, s) = \frac{e_0 + e_1 s}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (41)$$

Full expressions for $d_0, d_1, d_2, d_3, d_4, e_0$ and e_1 in terms of the primary rate constants of the kinetic model are given in Appendix D. Inverse transform of equation (39), (40) and (41) gives the mathematical expression for $P_4(j, t)$, $P_1(j, t)$ and $P_5(j, t)$.

Substituting the inverse Laplace transforms of (39), (40) and (41) into the equations (36), (37) and (38) respectively, we get the following distributions of the conditional dwell time:

$$\begin{aligned}
\Psi_{-+}(t) = & \left[\frac{(d_0 - d_1\omega_1 + d_2\omega_1^2 - d_3\omega_1^3 + d_4\omega_1^4)k_4}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\
& + \left[\frac{(d_0 - d_1\omega_2 + d_2\omega_2^2 - d_3\omega_2^3 + d_4\omega_2^4)k_4}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\
& + \left[\frac{(d_0 - d_1\omega_3 + d_2\omega_3^2 - d_3\omega_3^3 + d_4\omega_3^4)k_4}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\
& + \left[\frac{(d_0 - d_1\omega_4 + d_2\omega_4^2 - d_3\omega_4^3 + d_4\omega_4^4)k_4}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\
& + \left[\frac{(d_0 - d_1\omega_5 + d_2\omega_5^2 - d_3\omega_5^3 + d_4\omega_5^4)k_4}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \quad (42)
\end{aligned}$$

$$\begin{aligned}
\Psi_{--}(t) = & \left[\frac{k_x k_{-1} k_{-2} k_{-3} k_{-4}}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\
& + \left[\frac{k_x k_{-1} k_{-2} k_{-3} k_{-4}}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\
& + \left[\frac{k_x k_{-1} k_{-2} k_{-3} k_{-4}}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\
& + \left[\frac{k_x k_{-1} k_{-2} k_{-3} k_{-4}}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\
& + \left[\frac{k_x k_{-1} k_{-2} k_{-3} k_{-4}}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \quad (43)
\end{aligned}$$

$$\begin{aligned}
\Psi_{-x}(t) = & \left[\frac{(e_0 - e_1\omega_1)k_{exo}}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\
& + \left[\frac{(e_0 - e_1\omega_2)k_{exo}}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\
& + \left[\frac{(e_0 - e_1\omega_3)k_{exo}}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\
& + \left[\frac{(e_0 - e_1\omega_4)k_{exo}}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\
& + \left[\frac{(e_0 - e_1\omega_5)k_{exo}}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \quad (44)
\end{aligned}$$

where $\omega_1, \omega_2, \omega_3, \omega_4$ and ω_5 are the roots of the equation (34).

3.2.3 Calculation of $\Psi_{x+}, \Psi_{x-}, \Psi_{xx}$

Now we consider the case where DNA polymerase has arrived at site i by making an exonuclease cleavage. The initial condition

$$P_5(j, 0) = 1 \text{ and } P_1(j, 0) = P_2(j, 0) = P_3(j, 0) = P_4(j, 0) = 0 \quad (45)$$

ensures that previous mechanical step is an exonuclease cleaving. Now we define following distributions of conditional dwell time

$$\Psi_{x+}(t) = P_4(j, t)k_4|_{[P_5(j,0)=1, P_1(j,0)=P_2(j,0)=P_3(j,0)=P_4(j,0)=0]} \quad (46)$$

$$\Psi_{x-}(t) = P_1(j, t)k_{-4}|_{[P_5(j,0)=1, P_1(j,0)=P_2(j,0)=P_3(j,0)=P_4(j,0)=0]} \quad (47)$$

$$\Psi_{xx}(t) = P_5(j, t)k_{exo}|_{[P_5(j,0)=1, P_1(j,0)=P_2(j,0)=P_3(j,0)=P_4(j,0)=0]} \quad (48)$$

After applying the above initial condition in equation 22, we get

$$\tilde{P}_5(j, s) = \frac{f_4 s^4 + f_3 s^3 + f_2 s^2 + f_1 s + f_0}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (49)$$

$$\tilde{P}_4(j, s) = \frac{k_1 k_2 k_3 k_p}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (50)$$

$$\tilde{P}_1(j, s) = \frac{g_3 s^3 + g_2 s^2 + g_1 s + g_0}{\alpha s^5 + \beta s^4 + \gamma s^3 + \delta s^2 + \epsilon s + \zeta} \quad (51)$$

The expressions for $f_0, f_1, f_2, f_3, f_4, g_0, g_1, g_2$ and g_3 are given in Appendix D. The values of $P_4(j, t)$, $P_1(j, t)$ and $P_5(j, t)$ are obtained from the inverse Laplace transform of the (49), (50) and (51). After inserting the values of $P_4(j, t)$, $P_1(j, t)$ and $P_5(j, t)$ in equations (46), (47) and (48), we get the exact analytical expression for $\Psi_{x+}(t)$, $\Psi_{x-}(t)$ and $\Psi_{xx}(t)$.

$$\begin{aligned} \Psi_{xx}(t) = & \left[\frac{(f_0 - f_1 \omega_1 + f_2 \omega_1^2 - f_3 \omega_1^3 + f_4 \omega_1^4)k_{exo}}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\ & + \left[\frac{(f_0 - f_1 \omega_2 + f_2 \omega_2^2 - f_3 \omega_2^3 + f_4 \omega_2^4)k_{exo}}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\ & + \left[\frac{(f_0 - f_1 \omega_3 + f_2 \omega_3^2 - f_3 \omega_3^3 + f_4 \omega_3^4)k_{exo}}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\ & + \left[\frac{(f_0 - f_1 \omega_4 + f_2 \omega_4^2 - f_3 \omega_4^3 + f_4 \omega_4^4)k_{exo}}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\ & + \left[\frac{(f_0 - f_1 \omega_5 + f_2 \omega_5^2 - f_3 \omega_5^3 + f_4 \omega_5^4)k_{exo}}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \end{aligned} \quad (52)$$

$$\begin{aligned} \Psi_{x+}(t) = & \left[\frac{k_1 k_2 k_3 k_4 k_p}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\ & + \left[\frac{k_1 k_2 k_3 k_4 k_p}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\ & + \left[\frac{k_1 k_2 k_3 k_4 k_p}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\ & + \left[\frac{k_1 k_2 k_3 k_4 k_p}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\ & + \left[\frac{k_1 k_2 k_3 k_4 k_p}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \end{aligned} \quad (53)$$

$$\begin{aligned}
\Psi_{x-}(t) = & \left[\frac{(g_0 - g_1\omega_1 + g_2\omega_1^2 - g_3\omega_1^3)k_{-4}}{(\omega_1 - \omega_2)(\omega_1 - \omega_3)(\omega_1 - \omega_4)(\omega_1 - \omega_5)} \right] e^{-\omega_1 t} \\
& + \left[\frac{(g_0 - g_1\omega_2 + g_2\omega_2^2 - g_3\omega_2^3)k_{-4}}{(\omega_2 - \omega_1)(\omega_2 - \omega_3)(\omega_2 - \omega_4)(\omega_2 - \omega_5)} \right] e^{-\omega_2 t} \\
& + \left[\frac{(g_0 - g_1\omega_3 + g_2\omega_3^2 - g_3\omega_3^3)k_{-4}}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)(\omega_3 - \omega_4)(\omega_3 - \omega_5)} \right] e^{-\omega_3 t} \\
& + \left[\frac{(g_0 - g_1\omega_4 + g_2\omega_4^2 - g_3\omega_4^3)k_{-4}}{(\omega_4 - \omega_1)(\omega_4 - \omega_2)(\omega_4 - \omega_3)(\omega_4 - \omega_5)} \right] e^{-\omega_4 t} \\
& + \left[\frac{(g_0 - g_1\omega_5 + g_2\omega_5^2 - g_3\omega_5^3)k_{-4}}{(\omega_5 - \omega_1)(\omega_5 - \omega_2)(\omega_5 - \omega_3)(\omega_5 - \omega_4)} \right] e^{-\omega_5 t} \quad (54)
\end{aligned}$$

where $\omega_1, \omega_2, \omega_3, \omega_4$ and ω_5 are the roots of the equation (34).

The distributions of the conditional dwell times Ψ_{mn} , except Ψ_{xx} , are plotted for a few typical values of the parameters in figs.3 and 4. Since Ψ_{xx} is independent of the tension F , it has not been drawn graphically. We have also presented our numerical data, obtained from direct computer simulation, for the distributions plotted in fig.3. Each of these distributions is a sum of several exponentials. Therefore, in general, these distributions are expected to peak at a nonzero value of time t . However, some of the distributions in fig.3 and 4 appear as a single exponential. This single-exponential like appearance is an artefact of the parameters chosen for plotting these curves although, in reality, the full distributions are sum of several exponentials.

An interesting feature of the distributions plotted in figs.3 and 4 is a non-monotonic variation of the probability of the most probable conditional dwell times with increasing F (see, for example, Ψ_{++} and Ψ_{--}). This trend of variation is a consequence of the nonmonotonic variation of $\Delta\Phi$ with F (see fig.5).

4 Summary and conclusion

DNA replication is carried out by DNAP which operates as a molecular motor utilizing the template DNA strand as its track. In this paper we have presented a theoretical model for DNA replication that allows systematic investigation of the pol and exo activities as well as their coupling. More specifically, the situation considered here mimics an *in-vitro* experiment where a tension is applied on the template strand throughout the replication process. We have calculated the effect of the tension on the average speed of replication, capturing the effects of both the pol and exo activities of the same DNAP. Our theoretical results are in good qualitative agreement with the results of single molecule experiments reported in the literature.

However, the intrinsic fluctuations in the pol and exo processes contain some additional information which cannot be extracted from average properties. Distributions of various conditional dwell times defined here characterize these fluctuations. In contrast to all the molecular motors whose dwell time distributions have been investigated earlier, DNAP is characterized by 9 distinct conditional dwell times because of the interplay of its pol and exo activities. Each of these conditional dwell times can be identified with an appropriate first-passage time. Therefore, using the methods of calculation of the distributions of first-passage times, we derive exact analytical expressions for all these conditional dwell times

for the DNAP motor. These expressions explicitly show the effects of the template tension on the nature of the distributions. We believe that, in principle, these distributions can be measured in single molecule experiments [33]. But, to our knowledge, these have not been reported so far in the literature although both the pol and exo activities of the DNAP have been studied extensively [34]. We hope our model and results will motivate experiments to study the unexplored stochastic features of the kinetics of one of the most important genetic processes, namely DNA replication driven by DNAP. Understanding this kinetics will throw light on the propagation of life from one generation to the next.

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Appendix A

Here the parameters with subscripts “1” and “2” correspond to ssDNA and dsDNA, respectively. Let $b_i(F)$ ($i = 1, 2$) denote the average equilibrium projections of base pair in the direction of the applied force F . Suppose, $\Phi_i(F)$ ($i = 1, 2$) are the corresponding free energies. Then, for a given force F , the free energy difference between single base-pair of dsDNA and ssDNA can be expressed as [22]

$$\Delta\Phi(F) = \Phi_2(F) - \Phi_1(F) = - \int_0^F (b_2(F') - b_1(F')) dF' \quad (55)$$

where the right-hand side can be evaluated if the functions $b_i(F)$ are known.

For the freely jointed chain (FJC) model of DNA, is established.

$$b_i(F) = \left[\coth\left(\frac{2FA_i}{K_BT}\right) - \frac{K_BT}{2FA_i} \right] \left(1 + \frac{F}{K_i} \right) b_i^{max} \quad (56)$$

where K_i , A_i and b_i^{max} are, respectively, the elastic modulus, the persistence length and the average length of a base pair in the absence of any force.

Inserting the expression (56) into the equation (55) we numerically compute the free energy difference between single base pair of dsDNA and that of ssDNA for the given force F . In figure 5 we plot $\Delta\Phi$ against the tension F . The numerical values of the parameters that we use for this computation are given in the table 2. Since $\Delta\Phi$ is assumed to be independent of F beyond $f = 61$ pN; this captures the situation encountered in the *in-vitro* experiment of Wuite et al.[17].

Appendix B

$$x_1 = 1 \quad (57)$$

$$x_2 = \frac{k_1 + x_3 k_{-2}}{k_{-1} + k_2} \quad (58)$$

$$x_3 = \frac{k_2 k_1 (k_4 + k_{-3}) + k_{-3} k_{-4} (k_{-1} + k_2)}{(k_4 + k_{-3})(k_{-1} + k_2)(k_{-2} + k_3) - k_{-3} k_3 (k_{-1} + k_2) - k_2 k_{-2} (k_4 + k_{-3})} \quad (59)$$

$$x_4 = \frac{k_{-4} + x_3 k_3}{k_4 + k_{-3}} \quad (60)$$

Parameter values	
b_1^{max}	.58 nm
b_2^{max}	.34 nm
A_1	.7 nm
A_2	50 nm
K_1	900 pN
K_2	1000 pN

Table 2: Numerical values of the relevant parameters used for the computation of $\Delta\Phi$ using equation (55) and (56)

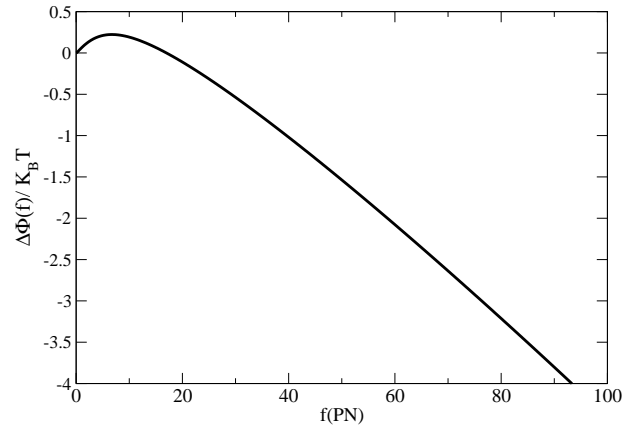


Figure 5: Free energy difference between dsDNA and ssDNA is plotted against the mechanical force applied on them

$$x_5 = \frac{k_x}{k_p} \quad (61)$$

Appendix C

$$\alpha = 1 \quad (62)$$

$$\beta = k_1 + k_2 + k_3 + k_4 + k_{-1} + k_{-2} + k_{-3} + k_{-4} + k_p + k_x + k_{exo} \quad (63)$$

$$\begin{aligned} \gamma = & k_1 k_2 + k_1 k_3 + k_2 k_3 + k_1 k_4 + k_2 k_4 + k_3 k_4 + k_{exo}(k_1 + k_2 + k_3 + k_4 \\ & + k_x + k_{-1} + k_{-2} + k_{-3} + k_{-4}) + k_p(k_1 + k_2 + k_3 + k_4 + k_{-1} + k_{-2} \\ & + k_{-3} + k_{-4}) + k_x(k_2 + k_3 + k_4 + k_{-1} + k_{-2} + k_{-3}) + k_{-1} k_{-2} \\ & + k_{-1} k_{-3} + k_{-2} k_{-3} + k_{-1} k_{-4} + k_{-2} k_{-4} + k_{-3} k_{-4} + k_1(k_{-2} + k_{-3}) \\ & + k_2(k_{-3} + k_{-4}) + k_3(k_{-1} + k_{-4}) + k_4(k_{-1} + k_{-2} + k_{-4}) \end{aligned} \quad (64)$$

$$\begin{aligned} \delta = & k_1 k_2 k_3 + k_1 k_2 k_4 + k_1 k_3 k_4 + k_2 k_3 k_4 + k_{-1} k_{-2} k_{-3} + k_{-1} k_{-2} k_{-4} \\ & + k_{-1} k_{-3} k_{-4} + k_{-2} k_{-3} k_{-4} + k_{exo}(k_1 k_2 + k_1 k_3 + k_2 k_3 + k_1 k_4 + k_2 k_4 \\ & + k_3 k_4 + k_{-1} k_{-2} + k_{-1} k_{-3} + k_{-2} k_{-3} + k_{-1} k_{-4} + k_{-2} k_{-4} + k_{-3} k_{-4}) \\ & + k_{exo} k_x(k_2 + k_3 + k_4 + k_{-1} + k_{-2} + k_{-3}) + k_{exo}(k_3 k_{-1} + k_4 k_{-1} + k_1 k_{-2} \\ & + k_4 k_{-2} + k_1 k_{-3} + k_2 k_{-3} + k_2 k_{-4} + k_3 k_{-4} + k_4 k_{-4}) + k_p(k_1 k_2 + k_1 k_3 \\ & + k_2 k_3 + k_1 k_4 + k_2 k_4 + k_3 k_4 + k_{-1} k_{-2} + k_{-1} k_{-3} + k_{-2} k_{-3} + k_{-1} k_{-4} \\ & + k_{-2} k_{-4} + k_{-3} k_{-4} + k_3 k_{-1} + k_4 k_{-1} + k_1 k_{-2} + k_4 k_{-2} + k_1 k_{-3} + k_2 k_{-3} \\ & + k_2 k_{-4} + k_3 k_{-4} + k_4 k_{-4}) + k_x(k_2 k_3 + k_2 k_4 + k_3 k_4 + k_{-1} k_{-3} + k_{-2} k_{-3} \\ & + k_{-1} k_{-2} + k_3 k_{-1} + k_4 k_{-1} + k_4 k_{-2} + k_2 k_{-3}) + k_3 k_4 k_{-1} + k_1 k_4 k_{-2} \\ & + k_4 k_{-1} k_{-2} + k_1 k_2 k_{-3} + k_1 k_{-2} k_{-3} + k_2 k_3 k_{-4} + k_2 k_4 k_{-4} + k_3 k_4 k_{-4} \\ & + k_3 k_{-1} k_{-4} + k_4 k_{-1} k_{-4} + k_4 k_{-2} k_{-4} + k_2 k_{-3} k_{-4} \end{aligned} \quad (65)$$

$$\begin{aligned} \epsilon = & k_1 k_2 k_3 k_4 + k_{-1} k_{-2} k_{-3} k_{-4} + k_{exo}(k_1 k_2 k_3 + k_1 k_2 k_4 + k_1 k_3 k_4 + k_2 k_3 k_4 \\ & + k_{-1} k_{-2} k_{-3} + k_{-1} k_{-2} k_{-4} + k_{-1} k_{-3} k_{-4} + k_{-2} k_{-3} k_{-4}) + k_{exo} k_x(k_2 k_3 \\ & + k_2 k_4 + k_3 k_4 + k_3 k_{-1} + k_4 k_{-1} + k_4 k_{-2} + k_{-1} k_{-2} + k_2 k_{-3} + \\ & + k_{-1} k_{-3} + k_{-2} k_{-3}) + k_{exo}(k_3 k_4 k_{-1} + k_1 k_4 k_{-2} + k_4 k_{-1} k_{-2} + k_1 k_2 k_{-3} \\ & + k_1 k_{-2} k_{-3} + k_2 k_3 k_{-4} + k_2 k_4 k_{-4} + k_3 k_4 k_{-4} + k_3 k_{-1} k_{-4} + k_4 k_{-1} k_{-4} \\ & + k_4 k_{-2} k_{-4} + k_2 k_{-3} k_{-4}) + k_p(k_1 k_2 k_3 + k_1 k_2 k_4 + k_1 k_3 k_4 + k_2 k_3 k_4 \\ & + k_{-1} k_{-2} k_{-3} + k_{-1} k_{-2} k_{-4} + k_{-1} k_{-3} k_{-4} + k_{-2} k_{-3} k_{-4} + k_3 k_4 k_{-1} \\ & + k_1 k_4 k_{-2} + k_4 k_{-1} k_{-2} + k_1 k_2 k_{-3} + k_1 k_{-2} k_{-3} + k_2 k_3 k_{-4} + k_2 k_4 k_{-4} \\ & + k_3 k_4 k_{-4} + k_3 k_{-1} k_{-4} + k_4 k_{-2} k_{-4} + k_2 k_{-3} k_{-4} + k_4 k_{-1} k_{-4}) + k_x(k_2 k_3 k_4 \\ & + k_{-1} k_{-2} k_{-3} + k_3 k_4 k_{-1} + k_4 k_{-1} k_{-2}) + k_4 k_{-1} k_{-2} k_{-3} + k_3 k_4 k_{-1} k_{-4} \\ & + k_2 k_3 k_4 k_{-4} \end{aligned} \quad (66)$$

$$\begin{aligned} \zeta = & k_{exo}(k_1 k_2 k_3 k_4 + k_2 k_3 k_4 k_x + k_3 k_4 k_x k_{-1} + k_4 k_x k_{-1} k_{-2} + k_x k_{-1} k_{-2} k_{-3} \\ & + k_2 k_3 k_4 k_{-4} + k_3 k_4 k_{-1} k_{-4} + k_4 k_{-1} k_{-2} k_{-4} + k_{-1} k_{-2} k_{-3} k_{-4}) \\ & + k_p(k_1 k_2 k_3 k_4 + k_2 k_3 k_4 k_{-4} + k_3 k_4 k_{-1} k_{-4} + k_4 k_{-1} k_{-2} k_{-4} \\ & + k_{-1} k_{-2} k_{-3} k_{-4}) \end{aligned} \quad (67)$$

Appendix D

$$a_0 = k_1 k_2 k_3 (k_{exo} + k_p) \quad (68)$$

$$a_1 = k_1 k_2 k_3 \quad (69)$$

$$b_0 = (k_{exo} + k_p)(k_2 k_3 k_4 + k_3 k_4 k_{-1} + k_4 k_{-1} k_{-2} + k_{-1} k_{-2} k_{-3}) \quad (70)$$

$$\begin{aligned} b_1 = & k_2 k_3 k_4 + k_3 k_4 k_{-1} + k_4 k_{-1} k_{-2} + k_{-1} k_{-2} k_{-3} + k_{exo}(k_2 k_3 + k_2 k_4 \\ & + k_3 k_4 + k_{-1} k_{-2} + k_{-2} k_{-3} + k_{-3} k_{-1} + k_3 k_{-1} + k_4 k_{-1} + k_2 k_{-3} + k_4 k_{-2}) \\ & + k_p(k_2 k_3 + k_2 k_4 + k_3 k_4 + k_{-1} k_{-2} + k_{-2} k_{-3} + k_{-3} k_{-1} + k_3 k_{-1} + k_4 k_{-1} \\ & + k_4 k_{-2} + k_2 k_{-3}) \end{aligned} \quad (71)$$

$$\begin{aligned} b_2 = & k_2 k_3 + k_2 k_4 + k_3 k_4 + (k_{exo} + k_p)(k_2 + k_3 + k_4 + k_{-1} + k_{-2} + k_{-3}) \\ & + k_3 k_{-1} + k_4 k_{-1} + k_4 k_{-2} + k_{-1} k_{-2} + k_2 k_{-3} + k_{-1} k_{-3} + k_{-2} k_{-3} \end{aligned} \quad (72)$$

$$b_3 = k_2 + k_3 + k_4 + k_{exo} + k_p + k_{-1} + k_{-2} + k_{-3} \quad (73)$$

$$b_4 = 1 \quad (74)$$

$$c_0 = k_x(k_2 k_3 k_4 + k_{-1} k_{-2} k_{-3} + k_3 k_4 k_{-1} + k_4 k_{-1} k_{-2}) \quad (75)$$

$$\begin{aligned} c_1 = & k_x(k_2 k_3 + k_2 k_4 + k_3 k_4 + k_3 k_{-1} + k_4 k_{-1} + k_4 k_{-2} + k_{-1} k_{-2} + k_2 k_{-3} \\ & + k_{-1} k_{-3} + k_{-2} k_{-3}) \end{aligned} \quad (76)$$

$$c_2 = k_x(k_2 + k_3 + k_4 + k_{-1} + k_{-2} + k_{-3}) \quad (77)$$

$$c_3 = k_x \quad (78)$$

$$\begin{aligned} d_0 = & k_{exo}(k_1 k_2 k_3 + k_2 k_3 k_x + k_3 k_x k_{-1} + k_x k_{-1} k_{-2} + k_2 k_3 k_{-4} + k_3 k_{-1} k_{-4} \\ & + k_{-1} k_{-2} k_{-4}) + k_p(k_1 k_2 k_3 + k_2 k_3 k_{-4} + k_3 k_{-1} k_{-4} + k_{-1} k_{-2} k_{-4}) \end{aligned} \quad (79)$$

$$\begin{aligned} d_1 = & k_1 k_2 k_3 + k_{-1} k_{-2} k_{-4} + k_{exo}(k_1 k_2 + k_1 k_3 + k_2 k_3 + k_3 k_{-1} + k_1 k_{-2} \\ & + k_2 k_{-4} + k_3 k_{-4} + k_{-1} k_{-2} + k_{-1} k_{-4} + k_{-2} k_{-4}) + k_{exo} k_x(k_2 + k_3 \\ & + k_{-1} + k_{-2}) + k_p(k_1 k_2 + k_1 k_3 + k_2 k_3 + k_3 k_{-1} + k_1 k_{-2} + k_2 k_{-4} \\ & + k_3 k_{-4} + k_{-1} k_{-2} + k_{-1} k_{-4} + k_{-2} k_{-4}) + k_x(k_2 k_3 + k_3 k_{-1} + k_{-1} k_{-2}) \\ & + k_2 k_3 k_{-4} + k_3 k_{-1} k_{-4} \end{aligned} \quad (80)$$

$$\begin{aligned} d_2 = & k_1 k_2 + k_1 k_3 + k_2 k_3 + k_{exo}(k_1 + k_2 + k_3 + k_x + k_{-1} + k_{-2} + k_{-4}) \\ & + k_p(k_1 + k_2 + k_3 + k_{-1} + k_{-2} + k_{-4}) + k_x(k_2 + k_3 + k_{-1} + k_{-2}) \\ & + k_3 k_{-1} + k_1 k_{-2} + k_{-1} k_{-2} + k_2 k_{-4} + k_{-1} k_{-4} + k_{-2} k_{-4} + k_3 k_{-4} \end{aligned} \quad (81)$$

$$d_3 = k_1 + k_2 + k_3 + k_{exo} + k_p + k_x + k_{-1} + k_{-2} + k_{-4} \quad (82)$$

$$d_4 = 1 \quad (83)$$

$$e_0 = k_{-1}k_{-2}k_{-3}(k_{exo} + k_p) \quad (84)$$

$$e_1 = k_{-1}k_{-2}k_{-3} \quad (85)$$

$$\begin{aligned} f_0 = & k_1k_2k_3k_4 + k_{-1}k_{-2}k_{-3}k_{-4} + k_x(k_2k_3k_4 + k_3k_4k_{-1} + k_4k_{-1}k_{-2} \\ & + k_{-1}k_{-2}k_{-3}) + k_3k_4k_{-1}k_{-4} + k_4k_{-1}k_{-2}k_{-4} + k_2k_3k_4k_{-4} \end{aligned} \quad (86)$$

$$\begin{aligned} f_1 = & k_1k_2k_3 + k_1k_2k_4 + k_1k_3k_4 + k_2k_3k_4 + k_3k_4k_{-1} + k_1k_4k_{-2} + k_4k_{-1}k_{-2} \\ & + k_1k_2k_{-3} + k_1k_{-2}k_{-3} + k_{-1}k_{-2}k_{-3} + k_2k_3k_{-4} + k_2k_4k_{-4} + k_3k_4k_{-4} \\ & + k_3k_{-1}k_{-4} + k_4k_{-1}k_{-4} + k_4k_{-2}k_{-4} + k_{-1}k_{-2}k_{-4} + k_2k_{-3}k_{-4} \\ & + k_{-1}k_{-3}k_{-4} + k_{-2}k_{-3}k_{-4} + k_x(k_2k_3 + k_2k_4 + k_3k_4 + k_3k_{-1} + k_4k_{-1} \\ & + k_4k_{-2} + k_{-1}k_{-2} + k_2k_{-3} + k_{-1}k_{-3} + k_{-2}k_{-3}) \end{aligned} \quad (87)$$

$$\begin{aligned} f_2 = & k_1k_2 + k_1k_3 + k_2k_3 + k_1k_4 + k_2k_4 + k_3k_4 + k_3k_{-1} + k_4k_{-1} + k_1k_{-2} \\ & + k_4k_{-2} + k_{-1}k_{-2} + k_1k_{-3} + k_2k_{-3} + k_{-1}k_{-3} + k_{-2}k_{-3} + k_2k_{-4} \\ & + k_3k_{-4} + k_4k_{-4} + k_{-1}k_{-4} + k_{-2}k_{-4} + k_{-3}k_{-4} + k_x(k_2 + k_3 + k_4 \\ & + k_{-1} + k_{-2} + k_{-3}) \end{aligned} \quad (88)$$

$$f_3 = k_1 + k_2 + k_3 + k_4 + k_x + k_{-1} + k_{-2} + k_{-3} + k_{-4} \quad (89)$$

$$f_4 = 1 \quad (90)$$

$$g_0 = k_p(k_2k_3k_4 + k_3k_4k_{-1} + k_4k_{-1}k_{-2} + k_{-1}k_{-2}k_{-3}) \quad (91)$$

$$\begin{aligned} g_1 = & k_p(k_2k_3 + k_2k_4 + k_3k_4 + k_3k_{-1} + k_4k_{-1} + k_4k_{-2} + k_{-1}k_{-2} + k_2k_{-3} \\ & + k_{-1}k_{-3} + k_{-2}k_{-3}) \end{aligned} \quad (92)$$

$$g_2 = k_p(k_2 + k_3 + k_4 + k_{-1} + k_{-2} + k_{-3}) \quad (93)$$

$$g_3 = k_p \quad (94)$$

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